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Null Mutation of *AtCUL1* Causes Arrest in Early Embryogenesis in *Arabidopsis*

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The SCF (for *S*KP1, *C*ullin/CDC53, *F*-box protein) ubiquitin ligase targets a number of cell cycle regulators, transcription factors, and other proteins for degradation in yeast and mammalian cells. Recent genetic studies demonstrate that plant F-box proteins are involved in auxin responses, jasmonate signaling, flower morphogenesis, photocontrol of circadian clocks, and leaf senescence, implying a large spectrum of functions for the SCF pathway in plant development. Here, we present a molecular and functional characterization of plant cullins. The *Arabidopsis* genome contains 11 cullin-related genes. Complementation assays revealed that AtCUL1 but not AtCUL4 can functionally complement the yeast *cdc53* mutant. *Arabidopsis* mutants containing transfer DNA (T-DNA) insertions in the *AtCUL1* gene were shown to display an arrest in early embryogenesis. Consistently, both the transcript and the protein of the *AtCUL1* gene were found to accumulate in embryos. The AtCUL1 protein localized mainly in the nucleus but also weakly in the cytoplasm during interphase and colocalized with the mitotic spindle in metaphase. Our results demonstrate a critical role for the SCF ubiquitin ligase in *Arabidopsis* embryogenesis.

INTRODUCTION

Ubiquitin conjugation to target proteins and subsequent degradation of the target proteins by the 26S proteasome play an important role in diverse cellular processes, including cell cycle regulation, stress responses, signal transduction, metabolic regulation, and cell differentiation (for review, see Hershko and Ciechanover, 1998). Three types of enzymes are involved sequentially in the ubiquitin-conjugation pathway: ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2), and ubiquitin ligase (E3). E1 catalyzes, in an ATP-dependent reaction, the formation of a ubiquitin adenylate that is then transferred to a conserved cysteine residue within the E1, resulting in the formation of a thiolester bond between the cysteinyl sulfhydryl group of E1 and the terminal carboxyl group of ubiquitin. The activated ubiquitin is subsequently transferred onto a cysteine residue within an E2. An E3 is typically required for the final transfer of the activated ubiquitin from the E2 to the lysine residue within the target protein, resulting in an isopeptide linkage between the C terminus of ubiquitin and the ϵ-lysyl group of the target protein. Once a polyubiquitin chain is assembled on a substrate, the substrate is then degraded by the 26S proteasome. The 26S proteasome is composed of two large subcomplexes, the 20S proteasome and the 19S regulatory cap. The plant counterpart appears to be similar in organization and structure to animal proteasome and prob-

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Accession numbers for complete cDNA sequence: AtCUL1 (AJ318017), AtCUL4 (AJ318018), AtCUL3A (AJ344252), NtCUL1 (AJ344533)

Abbreviations used: APC, anaphase-promoting complex; E1, ubiquitin-activating enzyme; E2, ubiquitin-conjugating enzyme; E3, ubiquitin ligase; GFP, green fluorescent protein; Hyg, hygromycin; Km, kanamycin; SCF, *S*KP1, *C*ullin/CDC53, *F*-box protein; T-DNA, transfer DNA.

ably functions in an analogous manner (Parmentier *et al.*, 1997; Fu *et al.*, 1998).

Work in yeast and mammals indicates that the specificity of the ubiquitin pathway derives from the activity of a specific E3 or E2/E3 combination. The SCF complex is a recently identified, and currently the best characterized, E3 complex that is composed of four majors subunits: cullin (CDC53 in yeast), SKP1, RBX1/ROC1, and an F-box protein (reviewed in Krek, 1998; Patton *et al.*, 1998a; Deshaies, 1999; Tyers and Jorgensen, 2000). Structure–function studies in yeast and mammals have demonstrated that cullin/CDC53 functions as a scaffold in assembling different subunits of the SCF complex as well as an E2 enzyme (e.g., CDC34). Different F-box proteins may be assembled onto the same core complex, forming different SCFs, which in turn catalyze the ubiquitination of different substrates .

Genetic studies have demonstrated the involvement of plant F-box proteins in a number of developmental and physiological processes (reviewed in Callis and Vierstra, 2000). The F-box protein UFO/FIM has an important role in regulating floral organ identity in *Arabidopsis* and *Antirrhinum* (Ingram *et al.*, 1997; Samach *et al.*, 1999). The *Arabidopsis* F-box proteins TIR1 and COI1 are essential for response to auxin and jasmonic acid, respectively (Ruegger *et al.*, 1998; Xie *et al.*, 1998). More recently, two closely related *Arabidopsis* F-box proteins, ZTL and FKF1, have been shown to be involved in the regulation of circadian rhythm (Nelson *et al.*, 2000; Somers *et al.*, 2000). The F-box protein EID1 is involved in phytochrome A–specific light signaling in *Arabidopsis* (Dieterle *et al.*, 2001). Finally, the F-box protein ORE9 seems to play a key role in natural and hormone-induced senescence processes (Woo *et al.*, 2001). For most of these F-box proteins, their interaction with ASK1 (the *Arabidopsis* SKP1-like protein) has been demonstrated by the yeast two-hybrid system and/or by immunoprecipitation assays, which implies their function through SCF complexes. The mutant *ask1–1* has been shown to be defective in homologous chromosome separation in male meiosis anaphase I (Yang *et al.*, 1999). Also, multiple aspects of vegetative and floral growth as well as response to auxin are affected in the *ask1–1* mutant (Gray *et al.*, 1999; Zhao *et al.*, 1999).

The *Arabidopsis* cullin AtCUL1 has been found in a complex containing TIR1 and ASK1 or ASK2 (Gray *et al.*, 1999). The modification of AtCUL1 by the ubiquitin-related protein RUB1 has been demonstrated, and genetic studies revealed that the enzymes responsible for this RUB1-conjugation pathway are important for auxin response (del Pozo and Estelle, 1999a; Dharmasiri and Estelle, 2002). Recently, the COP9 signalosome, first identified in *Arabidopsis* as a negative regulator of photomorphogenesis, has been shown to promote the removal of RUB1/NEDD8 from cullins (Lyapina *et al.*, 2001; Schwechheimer *et al.*, 2001; Zhou *et al.*, 2001). Strikingly, an increase in RUB1-modified AtCUL1 by knockdown of COP9 signalosome activity has the same effect on auxin response as a decrease in the amount of modified cullin. Schwechheimer *et al.* (2001) suggested that the RUB1 conjugation and deconjugation cycle is important for this process. An AMP-activated protein kinase SnRK has been demonstrated to interact with the SCF complex through binding with ASK1 (Farràs *et al.*, 2001). The function of this potential phosphorylation pathway on the SCF activity is currently unclear.

To study the role of AtCUL1 in *Arabidopsis* development, we identified T-DNA insertion *atcul1* mutants. In yeast, the *cdc53^{ts}* mutants fail to enter S phase because they are unable to degrade the S phase cyclin/CDK inhibitor SIC1 (Schwob *et al.*, 1994). The *Dictyostelium culA* mutants exhibit aggregation and morphogenesis defects (Mohanty *et al.*, 2001). In nematodes, the *cul1–1* mutants show hyperplasia of blast-cell lineages (Kipreos *et al.*, 1996). In mice, loss of the *CUL1* gene arrested embryogenesis before the onset of gastrulation (Dealy *et al.*, 1999; Wang *et al.*, 1999). Unlike animals, plants have multicellular haploid (gametophyte) and multicellular diploid (sporophyte) stages in their life cycle. In addition, higher plants have a sedentary lifestyle; plant cells that are trapped within rigid walls divide and differentiate in place. Despite the existence of large collections of mutants that affect plant embryogenesis (Meinke, 1985), the molecular basis underlying the developmental steps leading to early embryo development remains poorly understood. In this study, we show that null mutations in *AtCUL1* cause arrest before the first cell division of both embryo and endosperm cells, which originate from a double-fertilization event in which two sperm nuclei fuse with the egg cell and central cell nuclei, respectively. This work provides new insights into the role of the SCF pathway in the control of plant cell division and embryogenesis.

MATERIALS AND METHODS

Yeast Strains and Vectors

The yeast strain *cdc53st* and the plasmid pJS161–53 carrying the *CDC53* gene were a generous gift from D. Lammer and J. Singer (Hutchinson Cancer Research Center, Washington, DC), and the vector p426TEF (Mumberg *et al.*, 1995) from A. Camasses (Institut de Physiologie, Strasbourg, France). The cDNAs covering the entire coding region of AtCUL1, NtCUL1, and AtCUL4 were cloned into the p426TEF vector by use of *Bam*HI–*Xho*I, *Spe*I–*Xho*I, and *Eco*RI–*Sal*I restriction enzyme sites, respectively.

Plant Materials

The *Arabidopsis* plants were of the Wassilewskija ecotype. Seeds were produced under greenhouse conditions. *Arabidopsis* and tobacco BY2 cell suspensions were maintained by weekly subculture as described by Glab *et al.* (1994) and Nagata *et al.* (1992), respectively.

Antibodies

Peptides containing the N-terminal 20 amino acids of AtCUL1 were synthesized, linked to KLH carrier proteins, and used to immunize rabbits. The antiserum was immunoaffinity purified against the same peptides bound to Sepharose matrix. The affinity-purified anti-peptide antibody (@AtCUL1) was diluted 1:4000 for Western blot analysis and 1:500 for immunolocalization. Antibodies against PSTAIRE and α-tubulin were purchased from Santa Cruz Biotechnology, Santa Cruz, CA, and Amersham Pharmacia Biotech, Arlington Heights, IL, and used as recommended.

Northern and Western Analysis

Total RNAs and proteins were prepared from *Arabidopsis* plants and suspension-cultured cells. Northern and Western blot analyses were performed as described previously (Criqui *et al.*, 2000).

Plant Vectors

Schematic representations of different plant vectors and sequences of oligonucleotides used in PCR amplification for vector constructions are shown in Figure 4.

An epitope of 10 histidines was fused to the N- and C-terminus of the AtCUL1 by PCR amplification of the *AtCUL1* cDNA by use of oligonucleotides P1 and P2, and P3 and P4, respectively. The amplified fragments were cloned into the *Bam*HI and *Sac*I restriction sites of the binary vector pBI121.1 (Clontech, Cambridge, UK), resulting in pBI-HisAtCUL1 and pBI-AtCUL1His.

The whole coding region of the *AtCUL1* cDNA was PCR-amplified by use of oligonucleotides P5 and P6 and subsequently cloned into the *Xho*I and *Nco*I sites of pSK-GFP (Criqui *et al.*, 2000), resulting in pSKAtCUL1-GFP. Similarly, the PCR-amplified fragment of the *AtCUL1* cDNA by use of oligonucleotides P3 and P7 was cloned into pKS-GFP by use of *Bam*HI and *Spe*I sites, resulting in pKS-GFPAtCUL1. After confirmation by sequencing of the *AtCUL1* sequences and its in-frame fusion with green fluorescent protein (GFP), the *Xho*I-*Spe*I DNA fragments encoding the chimeric AtCUL1-GFP and GFP-AtCUL1 proteins were subcloned into the glucocorticoid-inducible vector pTA7002 (Aoyama and Chua, 1997), resulting in pTA-AtCUL1GFP and pTA-GFPAtCUL1, respectively.

The *Arabidopsis* BAC T10P11 containing the *AtCUL1* gene was received from the Genome Sequencing Center of the Cold Spring Harbor Laboratory. The region spining the *AtCUL1* gene was PCR-amplified by use of oligonucleotides P7 and P8. The resulting PCR fragment was digested with *Eco*RI and *Spe*I and subsequently cloned into the *Eco*RI- and *Xba*I-digested pBinHyg-TX vector (Gatz, 1995), resulting in pBH-*AtCUL1*. Sequence analysis of the cloned fragment revealed that PCR amplification caused three T -to-C substitutions. Luckily, these substitutions were located at positions nonessential for the expression of AtCUL1.

These different plant vectors were transferred by electroporation into *Agrobacterium*, and the resulting strains were used in plant transformation.

Plant Transformation and Transgene Expression Analysis

Transgenic *Arabidopsis* plants were obtained by *Agrobacterium*-mediated transformation by the floral dip method (Bechtold *et al.*, 1993; Clough and Bent, 1998). Tobacco plant transformation and the establishment of transgenic BY2 cell lines were as previously described (Shen, 2001b). The DEX induction for transgene expression and the confocal microscopy detection of GFP fluorescence were as previously described (Shen, 2001b).

Isolation of atcul1 *Mutants*

DNA pools of the *Arabidopsis* T-DNA insertion lines from the Versailles collection (Bechtold *et al.*, 1993) were screened for T-DNA insertion in the *AtCUL1* locus. Forward and reverse primers from the sequence of the *AtCUL1* gene were designed for PCR screening of the DNA pools by the combination of T-DNA left and right border–specific primers. PCR products were analyzed by Southern hybridization with the *AtCUL1* cDNA and the T-DNA probes. PCR fragments hybridized with both probes were further confirmed by sequencing.

Segregation Analysis

Seeds were surface-sterilized and plated onto medium supplemented with kanamycin (Km) (50 mg/L) (half-strength Murashige and Skoog salts, 1% sucrose, 0.9% agar, pH 5.7). After 2 d at 4°C, the seeds were grown under 12 h light/12 h dark cycles at 22°C. The Km phenotype (resistant or sensitive) was scored after 2 weeks.

Intact Silique Analysis and Whole-Mount Preparation of Ovules

Siliques were dissected fresh or after fixation in an ethanol/acetic acid (9:1) solution. Seeds (ovules) were removed from fixed siliques, cleared for 10 min to 2 h in Hoyer's solution (chloral hydrate/gum arabic/glycerol/water [100:7.5:5:30 g]), and imaged by use of Nomarski optics.

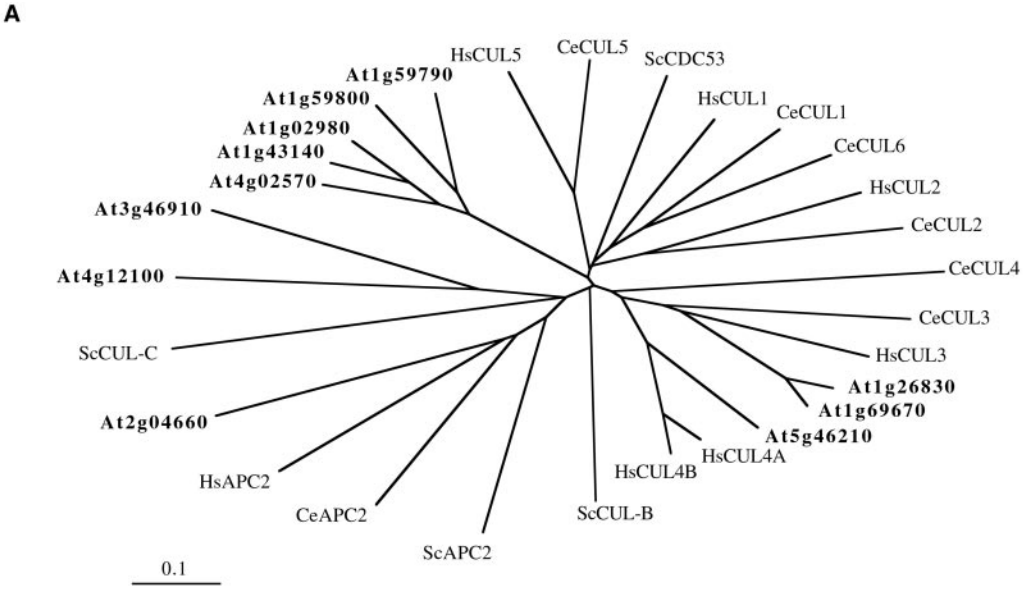
Immunofluorescence Staining and In Situ Hybridization

Tobacco BY2 cells as well as *Arabidopsis* suspension cells were fixed in 3.7% paraformaldehyde as described (Proust *et al.*, 1999). Inflorescences and siliques of *Arabidopsis* plants were fixed in 4% paraformaldehyde and embedded in paraffin wax, and 10-μm sections were prepared for immunolabeling and for in situ hybridization according to Jackson (1991). Immunolabeling was performed as described by Schmit *et al.* (1996). The sense and antisense *AtCUL1* probes for in situ hybridization were prepared by use of the DIG RNA Labeling Kit (Roche; Catalog No. 1175025), and hybridizations were performed as described by Jackson (1991).

RESULTS

Arabidopsis Contains Multiple Putative Cullins

The cullin family encompasses at least six genes in humans and in *Caenorhabditis elegans*. The genome sequence of *Arabidopsis* has been determined (*Arabidopsis* genome initiative, 2000). A sequence similarity search reveals that the *Arabidopsis* genome contains 11 cullin-related genes that together with other eukaryotic members can be classified into distinct, distantly related groups (Figure 1A). The APC2 group member contains a cullin homology region but is a subunit of the anaphase-promoting complex (APC), an E3 enzyme responsible for ubiquitination of mitotic regulators (for review, see Zachariae and Nasmyth, 1999). The APC-mediated destruction box pathway also seems to be conserved in plants (Genschik *et al.*, 1998; Criqui *et al.*, 2000). The ubiquitin-ligase complexes containing CUL3, CUL4, CUL5, and CeCUL6 are poorly characterized, although CUL3 has been demonstrated to be involved in the degradation of cyclin E (Singer *et al.*, 1999). The HsCUL2 protein functions in a ubiquitin-ligase complex containing the VHL tumor suppressor protein elongin-B (a ubiquitin-like protein), RBX1/ROC1, and elongin-C (a SKP1 functional homologue) that recruits a SOCS-box containing protein (reviewed in Tyers and Jorgensen, 2000; Ivan and Kaelin, 2001). Also, CeCUL2 is not functionally redundant with CeCUL1 (Feng *et al.*, 1999). The mammalian and nematode CUL1 show the highest homology with ScCDC53 and form SCF complexes with similar partners: SKP1, ROC1/RBX1, and an F-box protein (reviewed in Krek, 1998; Deshaies, 1999; Tyers and Jorgensen, 2000). The AtCUL1 (At4g02570) protein, together with four other *Arabidopsis* proteins (At1g43140, At1g02980, At1g59800, and At1g59790), are the closest orthologues of ScCDC53, but their sequence does not allow assignment to either the CUL1 or CUL2 group (Figure 1A). Among these *Arabidopsis* proteins, only AtCUL1 has been demonstrated to be expressed (del Pozo and Estelle, 1999b; Gray *et al.*, 1999; Farràs *et al.*, 2000); whether the other proteins are also expressed is currently unknown. In addition, two of them (At1g59800 and At1g59790) contain the conserved N-terminal region (Figure 1B), which is involved in the interaction



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At4g02570   -----MER-KTIDLEQGWDYMQTGITKLKRILEGLN-EPAFDSEQYMMLYTTIYRMCTQKPPHDYSQQLYDKYREAFEEYINSTVLPALREKHDEFMLRELFKRWSNHKVMVRWLSRFFY  113
At1g43140   ------MK-ELILLEEGWSVMKTGVAKLQRILEDLS-EPPFDPGQYINLYTIIYDMCLQQPPNDYSQELYNKYRGVVDHYNKETVLPSMREKHGEYMLRELVKRWANHKILVRWLSRFCF  112
At1g02980   -----MAK-KDSVLEAGWSVMEAGVAKLQKILEEVPDEPPFDPVQRMQLYTTVHNLCTQKPPNDYSQQIYDRYGGVYVDYNKQTVLPAIREKHGEYMLRELVKRWANQKILVRWLSHFFE  114
At1g59800   -----MVAPTEIKFEVEWSNIQQGFTKLIRMIEGES-EPAFNQEIMMMMHTATYRICAYK----NPQQLYDKYRELIENYAIQTVLPSLREKHDECMLRELAKRWNAHKLLVRLFSRRLV  110
At1g59790   MDSRRMSRPRQIKFEEGWSNIQKGITKLIRILEGEP-EPTFYFSECFKLYTIIYDMCVQR--SDYSQQLYEKYRKVIEDYTIQTVLPSLREKHDEDMLRELVKRWNNHKIMVKWLSKFFV  117

                    *:*     :          **: .* * ** *::* . :.::**:.********** *:.**:*:;    *:* :  ****** .:*:*:.:**** :** * :*:** ** :* .:.*: *
At4g02570   YLDRYFI--ARRSLPPLNEVGLTCFRDLVYNELHSKVKQAVIALVDKERERGEQIDRALLKNVLDIYVEIGMGQMERYEEDFESFMLQDTSSYYSRKASSWIQEDSCPDYMLKSEECLKKE  231
At1g43140   YLDRFYV--ARRGLPTLNDVGFTSFHDLVYQEIQSEAKDVLLALIHKEREGEQIDRTLVKNVIDVYCGNGVGQMVIYEEDFESFLLQDTASYYSRKASRWSQEDSCPDYMLKAEECLKLE  230
At1g02980   YLDRFY---A---------VGFISFRDLVYQELQSKAKDAVLALIHKEREGEQIDRALLKNVIDVYCGNGMGELVKYEEDFESFLLEDSASYYSRNASRWNQENSCPDYMIKAEESLRLE  222
At1g59800   YLDDSFL--SKKGLPSLREVGLNCFRDQVYREMQSMAAEAILALIHKEREGEQIDRELVRNVIDVFVENGMGTLKKYEEDFERLMLQDTASYYSSKASRWIQEESCLDYTLKPQQCLQRE  228
At1g59790   YIDRHLVRRSKIPIPSLDEVGLTCFLDLVYCEMQSTAKEVVIALIHKEREGEQIDRALVKNVLDIYVENGMGTLEKYEEDFESFMLQDTASYYSRKASRWTEEDSCPDYMIKVEECLKME  237

                    :*:**::***. :****.  ::  :::
At4g02570   RERVAHYLHSSSSEPKLVEKVQHELLVVFASQLLEKEHSGCRALLRDDKVDDLSRMYRLYHKILRGLEPVANIFKQHVTAEGNALVQQAEDTATNQVANTAS--VQEQVLIRKVIELHDKY  349
At1g43140   KERVTNYLHSTTEPKLVEKVQNELLVVVAKQLIENEHSGCLALLRDDKMGDLSRMYRLYRLIPQGLEPIADLFKQHVTAEGNALIKQAADAATNQDASAS------QVLVRKEIELHDKY  344
At1g02980   KERVTNYLHSTTEPKLVAKVQNELLVVVAKQLIENEHSGCRALLRDDKMDDLARMYRLYHPIPQGLDPVADLFKQHITVEGSALIKQATEAATDKAASTSGLKVQDQVLIRQLIDLHDKF  342
At1g59800   RERVTHYLHPTTEPKLFE-VRYGIIIWN-------------------------------------------------------------------------------------------  255
At1g59790   RERVTHYLHSITEPKLVEKIQNELLVMVTKNRLENEHSGFSALLRDDKKNDLSRIYRLYLPIPKRLGRVADLFKKHITEEGNALIKQADDKTTN----------------QLLIELHNKF  341

At4g02570   MVYVTECFQNHTLFHKALKEAFEIFCNKTVAGSSSAELLATFCDNILKK-GGSEKLSDEAIEDTLEKVVKLLAYISDKDLFAEFYRKKLARRLLFDRSANDDHERSILTKLKQQCGGQFT  468
At1g43140   MVYVDECFQKHSLFHKLLKEAFEVFCNKTVAGASSAEILATYCDNILKTRGGSEKLSDEATEITLEKVVNLLVYISDKDLFAEFYRKKQARRLLFDRSG----------------------  443
At1g02980   MVYVDECFQKHSLFHKALKEAFEVFCNKTVAGVSSAEILATYCDNILKTGGGIEKLENEDLELTLEKVVKLLVYISDKDLFAEFFRKKQARRLLFDRNGNDYHERSLLTKFKELLGAQFT  462
At1g59800   ------------------------------------------------------------------------------------------------------------------------  255
At1g59790   IVYVIECFQNHTLFHKVR-----------------------------------------------------VLFMCVYL-----FQQYYIE-------------------------------  374

                                                                                                                                  ▼
At4g02570   SKMEGMVTDLTLARENQNSFEDYLGSNPAANPGIDLTVTVLTTGFWPSYKSFDINLPSEMIKCVEVFKGFYETKTKHRKLTWIYSLGTCHINGKFDQKAIELIVSTYQAAVLLLFNTTDK  588
At1g43140   --IMKEVTDITLARELQTNFVDYLSANMTTKLGIDFTVTVLTTGFWPSYKTTDLNLPTEMVNCVEAFKVFYGTKTNSRRLSWIYSLGTCHILGKFEKKTMELVVSTYQAAVLLLFNNAER  561
At1g02980   SKMEGMLTDMTLAKEHQTNFVEFLSVNKTKKLGMDFTVTVLTTGFWPSYKTTDLNLPIEMVNCVEAFKAYYGTKTNSRRLSWIYSLGTCQLAGKFDKKTIEIVVTTYQAAVLLLFNNTER  582
At1g59800   ------------------------------------------------------------------------------------------------------------------------  255
At1g59790   ------------------------------------------------------------------------------------------------------------------------  374

At4g02570   LSYTEILAQLNLSHEDLVRLLHSLSCAKYKILLKEPNTKTVSQNDAFEFNSKFTDRMRRIKIPLPPVDERKKVVEDVDKDRRYAIDAAIVRIMKSRKVLGHQQLVSECVEQLSRMFKPDI  708
At1g43140   LSYTEISEQLNLSHEDLVRLLHSLSCLKYKILIKEPMSRTISKTDTFEFNSKFTDKMRKIRVPLPPMDERKKVVEDVDKDRRYAIDAALVRIMKSRKVLAHQQLVSECVEHLSKMFKPDI  681
At1g02980   LSYTEILEQLNLGHEDLARLLHSLSCLKYKILIKEPMSRNISNTDTFEFNSKFTDKMRRIRVPLPPMDERKKIVEDVDKDRRYAIDAALVRIMKSRKVLGHQQLVSECVEHLSKMFKPDI  702
At1g59800   ------------------------------------------------------------------------------------------------------------------------  255
At1g59790   ------------------------------------------------------------------------------------------------------------------------  374

At4g02570   KAIKKRMEDLITRDYLERDKENPNMFRYLA   738
At1g43140   KMIKKRIEDLINRDYLERDTENANTFKYVA   711
At1g02980   KMIKKRIEDLISRDYLERDTDNPNTFKYLA   732
At1g59800   ----------------------------   255
At1g59790   ----------------------------   374
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Figure 1. Sequence analysis of *Arabidopsis* cullin-related proteins. (A) Phylogenetic tree of the *Arabidopsis* proteins (bold letters), together with cullins and APC2 of *Saccharomyces cerevisiae*, *C. elegans*, and *Homo sapiens*, was established by use of ClustalW and TreeViewPPC programs. DDBJ/EMBL/GenBank accession numbers: Q12018 for ScCDC53, P53202 for ScCUL-B, NP__012488 for ScCUL-C, NP__013228 for ScAPC2, Q17389 for CeCUL-1, Q17390 for CeCUL-2, Q17391 for CeCUL-3, Q17392 for CeCUL-4, Q23639 for CeCUL-5, Q21346 for CeCUL-6, AAF99984 for CeAPC2, NP__003583 for HsCUL1, NP__003582 for HsCUL2, NP__003581 for HsCUL3, NP__003580 for HsCUL4A, AAK16812 for HsCUL4B, AAK07472 for HsCUL5, NP__037498 for HsAPC2, AAK76704 for At4g02570 (AtCUL1, this work), NP__175007 for At1g43140, NP__171797 for At1g02980, NP__176189 for At1g59800, NP__176188 for At1g59790, NP__174005 for At1g26830, NP__177125 for At1g69670, AJ318018 for At5g46210 (AtCUL4, this work), NP__178543 for At2g04660, NP__192947 for At4g12100, and NP__190275 for At3g46910. (B) Sequence alignment of AtCUL1 (At4g02570) and its most closely related *Arabidopsis* proteins was performed by use of ClustalX. Numbers refer to amino acid positions in the corresponding proteins. Consensus symbols on top of the alignment: * for the identical or conserved residues in all sequences; : and . for the conserved and semiconserved substitutions, respectively. The RBX1/ROC1 binding domain and the RUB1/NEDD8 conjugation site are indicated by a line and an arrow, respectively.

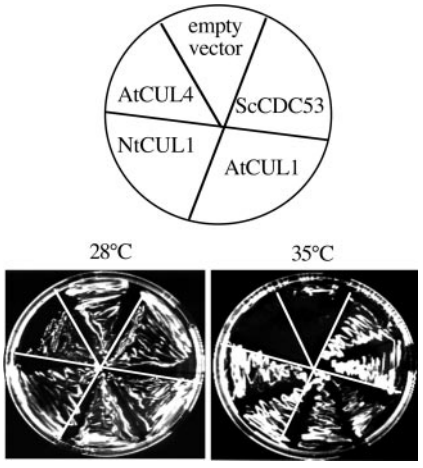

Figure 2. Complementation of the yeast *cdc53* mutant by plant cullins. The yeast temperature-sensitive mutant *cdc53^{ts}* was transformed with the empty vector or the vectors expressing CDC53, AtCUL1, NtCUL1, and AtCUL4, respectively. Individual transformants were plated on selective media and grown either at permissive (28°C) or at restrictive (35°C) temperature. Photographs were taken after 4 d.

with SKP1 (Patton *et al.*, 1998b; Wu *et al.*, 2000), but do not contain the conserved C-terminal region, which is required for interaction with RBX1/ROC1 and RUB1/NEDD8 modification (Furukawa *et al.*, 2000; Wu *et al.*, 2000).

AtCUL1 but Not AtCUL4 Complements the Yeast cdc53^{ts} *Mutant Phenotype*

To test whether plant cullins can functionally replace ScCDC53 to form active SCF complexes in yeast, we introduced the plant cullin cDNAs under the control of the TEF promoter (Mumberg *et al.*, 1995) into the yeast *cdc53^{ts}* mutant strain, carrying a temperature-sensitive mutation in the Sc*CDC53* gene. Transformants that expressed ScCDC53 (positive control) or AtCUL1 were able to grow at a restrictive temperature (35°C), whereas the negative control containing the empty vector was not (Figure 2). The tobacco orthologue of AtCUL1, the NtCUL1, was also able to complement the yeast mutant. AtCUL4 (At5g46210, Figure 1A), however, failed to complement, which in addition inhibited yeast growth even at a permissive temperature (28°C).

Expression of AtCUL1 *in* Arabidopsis *Plants and Suspension Cells*

RNA blot analysis shows that *AtCUL1* encodes a single transcript of ~2.5 kb, which is present in different organs of *Arabidopsis* plants (Figure 3A). Whereas the histone H4 transcripts show higher levels in organs containing actively dividing cells (such as flowers and young seedlings), the *AtCUL1* did not exhibit such specificity of expression, suggesting that the *AtCUL1* gene is not cell cycle regulated. In agreement with this assumption, the *AtCUL1* transcript was found at a relatively constant level during different growth phases of suspension-cultured cells (Figure 3B). Also, in the synchronized tobacco BY2 cells, the *NtCUL1* transcript was constantly present during different phases of the cell cycle (data not shown).

Antibodies directed against the N-terminal 20-amino-acid peptide of AtCUL1 were produced in rabbits and affinity purified against the antigen. Western blot analysis revealed that the antibodies specifically recognized AtCUL1 expressed in transgenic tobacco BY2 cells but not the endogenous tobacco cullins (Figure 3C). As described previously (del Pozo and Estelle, 1999b; Gray *et al.*, 1999), two predominant bands that migrate close together were detected in the total protein extract from *Arabidopsis* seedlings (Figure 3C). They correspond to unconjugated and RUB1-conjugated isoforms of AtCUL1 (del Pozo and Estelle, 1999b). Interestingly, in both transgenic tobacco BY2 cells expressing AtCUL1 (Figure 3C, lane 2) and *Arabidopsis* suspension-cultured cells (Figure 3B), only the unconjugated isoform was observed. Like its transcript, the AtCUL1 protein was present at a relatively constant level during different growth phases of suspension-cultured cells (Figure 3B).

AtCUL1 Is Localized in Nucleus, Cytoplasm, and Metaphase Spindles

To study the localization of AtCUL1, both GFP and immunolocalization technologies were used. Transgenic tobacco BY2 cell lines expressing the 10×his-tagged AtCUL1 or the GFP-fused AtCUL1 (Figure 4) as well as transgenic tobacco plants expressing the GFP-fused AtCUL1 were generated. In interphase cells, GFP-AtCUL1 as well as AtCUL1-GFP were localized mainly to nucleus and weakly to cytoplasm in transgenic BY2 cells as well as in transgenic plants (Figure 5, A–C). Immunolocalization in *Arabidopsis* cells confirms this pattern of AtCUL1 localization (Figure 5D). Control immunolocalization experiments using the anti- AtCUL1 preimmune serum, either with *Arabidopsis* (data not shown) or with tobacco BY2 cells constitutively expressing AtCUL1 (Figure 5E), did not show any detectable staining above background levels. In late G2 phase, a microtubule array called the preprophase band defines the future division plane of the plant cell. At this stage, AtCUL1 was still detected mainly in the nucleus and barely on the preprophase band (Figure 5F). Colocalization of AtCUL1 with mitotic spindle was observed at metaphase (Figure 5G). At telophase, AtCUL1 weakly colocalized with the phragmoplast (Figure 5H). On entrance into interphase, AtCUL1 localized primarily to the newly formed nucleus (Figure 5I).

Mutants of AtCUL1 *Show Reduced Inheritance in the Gametophyte and Embryonic Lethality*

Two T-DNA insertion lines, *atcul1–1* and *atcul1–2*, were identified by PCR screening of a total of 40,000 independent transgenic lines of the Versailles T-DNA collection. In *atcul1–1*, two copies of T-DNA were inserted head-to-head in the intron between the 17th and 18th exons (Figure 6). The insertion also caused a deletion of 25 nucleotides at the 5′ end of the intron and an addition of 13 nucleotides of unknown origin at the 5′ end junction between *AtCUL1* and the T-DNA. In *atcul1–2*, two copies of T-DNA were inserted head-to-head in the third exon, which also resulted in a small deletion of 16 nucleotides of the exon and the addition

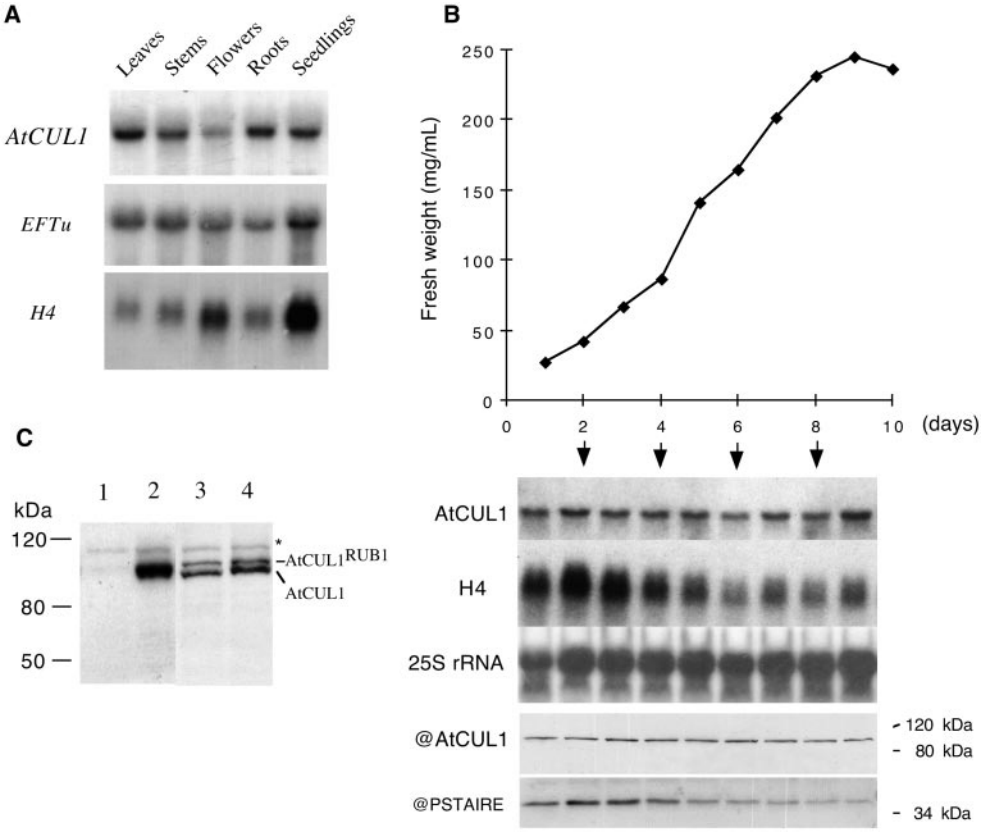

Figure 3. Northern and Western blot analyses of *AtCUL1* expression. (A) Total RNA was isolated from different organs of *Arabidopsis* plants, and Northern analysis was performed by successive hybridizations with different probes, as indicated. EFTu: elongation factor EF-1α; H4: histone H4. (B) Samples were taken at different days of subculture from an *Arabidopsis* cell suspension culture and used for fresh weight measurement and for RNA and protein analysis. Northern analysis was performed by successive hybridizations with the indicated probes. Western blots were performed with the antibodies against AtCUL1 (@AtCUL1) and the conserved CDK kinase motif PSTAIRE (@PSTAIRE). (C) Total proteins prepared from tobacco BY2 cells (lane 1), transgenic BY2 cells expressing 10×his-tagged AtCUL1 (lane 2), and 2-week-old *Arabidopsis* seedling of wild-type Wassilewskija genotype (lane 3) and Km-resistant *atcul1–1*$^{+/-}$ (see Table 1) genotype (lane 4) were Western blotted with @AtCUL1. The asterisk indicates an aspecific protein band, which cannot be competed by the AtCUL1 peptide (data not shown).

of 7 and 2 nucleotides at the 5′ and 3′ end junctions between *AtCUL1* and the T-DNA, respectively (Figure 6).

Heterozygous plants of the *atcul1–1* line appeared normal in morphology. A decreased level of AtCUL1 was barely evident in these heterozygous plants (Figure 3C). The T-DNA inserted in the *atcul1* mutants contains the chimeric *nptII* gene that confers Km resistance (Figure 6). Segregation tests for Km resistance on seeds produced by self-pollination of more than 60 individual *atcul1–1* plants revealed that homozygous *atcul1–1* plants could not be obtained. In addition, as shown in Table 1, the ratio of Km-resistant-to-Km-sensitive in self-progeny of individual heterozygous plants was significantly lower than the expected ratio of 3:1. The *atcul1–2* line behaved very similarly to *atcul1–1* (Table 1). To determine the inheritance of the *atcul1* mutations in the male and female gametophytes, reciprocal backcrosses of heterozygous mutant plants with the wild-type plants were performed. Genetic analysis of Km resistance in the F1 progeny revealed that the inheritance of both *atcul1–1* and *atcul1–2* mutations was reduced through both male and female gametes (Table 1). Together, these genetic studies reveal that mutations in the *AtCUL1* gene affect the development, viability, or function of both male and female gametophytes and that homozygous *atcul1* mutant embryos are aborted before seed production.

Mutations in AtCUL1 *Are Responsible for the Phenotype*

To confirm that *atcul1–1* and *atcul1–2* are alleles, crosses between plants that were heterozygous for the two mutations were performed. The resulting F1 progeny exhibited a ratio of Km-resistant-to-Km-sensitive similar to that of the self-progeny of either mutant (Table 1). PCR-amplification analysis revealed that the Km-resistant plants produced from the crosses were either *atcul1–1* or *atcul1–2* genotype but never both (data not shown). These results indicate that *atcul1–1* and *atcul1–2* are allelic mutations responsible for the mutant phenotype.

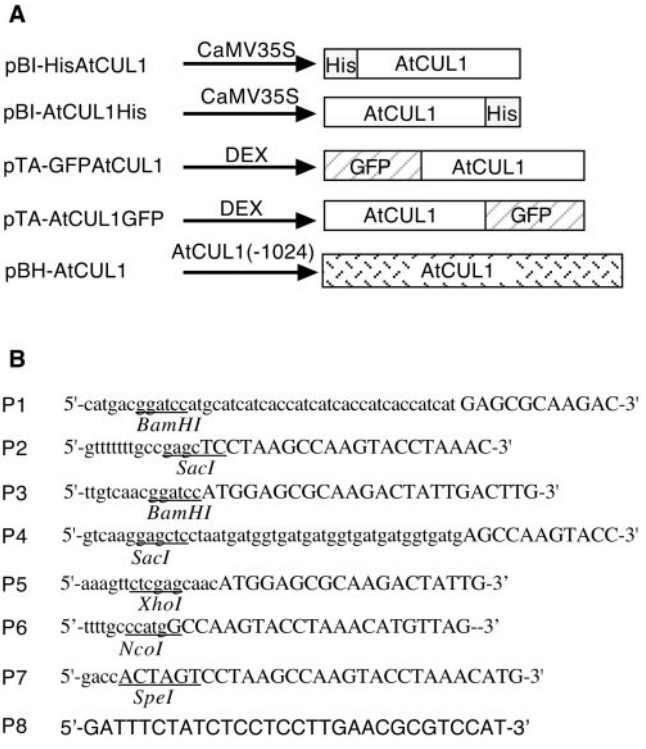

Figure 4. Vectors used for plant transformation. (A) Schematic representation of the genes inserted into different vectors. Arrows with lines represent the different promoter regions and open boxes the coding sequence of different proteins. (B) Oligonucleotides used in PCR amplification for plant vector construction. Nucleotides corresponding to the *AtCUL1* sequence are specified by capital letters. Restriction enzyme sites used in cloning are underlined.

To further confirm that the mutation of *AtCUL1* is responsible for the phenotype, genetic complementation was carried out. The first construct used carried the *AtCUL1* cDNA under the control of the CaMV 35S promoter and failed to rescue the mutant phenotype of *atcul1–1* (data not shown). A genomic fragment spanning from the −1024 base pairs upstream of the ATG to the stop codon of the *AtCUL1* gene was subsequently cloned into a vector carrying the *hpt* gene, which confers hygromycin (Hyg) resistance (pBH-*AtCUL1*, Figure 4). Hyg-resistant plants were obtained from transformation of heterozygous mutant plants, and their self-progeny were scored for Km resistance. Of six independent transformants of *atcul1–1* that produced Km-resistant progeny, three were found to display a ratio of Km-resistant-to-Km-sensitive of ~3:1 (one of them is shown in Table 1), as expected for rescue by the transgene. One transformant obtained on *atcul1–2* exhibited the rescued segregation phenotype as well (Table 1).

Mutants of AtCUL1 *Are Arrested Before the First Cell Divisions after Fertilization during Embryogenesis*

More than 50 siliques (with a total of more than 2000 ovules) on heterozygous *atcul1–1* plants were dissected after self-pollination. Among these, ~27% of embryos failed to develop (indicated by arrows), whereas the others developed normally into mature seeds (Figure 7A). A similar embryo-lethal phenotype was observed in the *atcul1–2* line and also in two other *atcul1* mutant alleles (H. Hellmann and M. Estelle, unpublished observations) recovered from the Wisconsin collection of T-DNA insertion lines. Differential interference contrast imaging of cleared ovules revealed that whereas the normal ovules contained zygotes that develop through the characteristic preglobular (Figure 7B), globular (Figure 7C), and heart (Figure 7D) stages into mature embryos, the arrested ovules do not contain developed zygotes (Figure 7, E–G). In addition, mutant ovules are missing the endosperm cells, which divide earlier and fill the space around the zygotes. The most advanced stage of an arrested ovule contained one zygote and one endosperm cell with either one or two nuclei (Figure 7, F and G) that were not properly separated from each other (Figure 7H).

The expression of the *AtCUL1* during embryogenesis was further analyzed. In situ hybridization showed that the *AtCUL1* antisense probe detected a strong signal in the embryo (Figure 8, B and C) but not the negative control sense probe (Figure 8A). Immunolocalization using the AtCUL1 antibody detected a strong staining in embryos, particularly in the nucleus (Figure 8, E and F). Such staining was not detected in the negative control by use of the preimmune serum (Figure 8D). These results together demonstrated high expression levels of *AtCUL1* transcript and protein in embryos, which further underscores the important function of AtCUL1 during embryogenesis.

DISCUSSION

Arabidopsis Contains Functionally Distinct Cullins

All of the SCF subunits, including the cullins, are highly conserved from yeast to mammals, suggesting a common biochemical mechanism of protein ubiquitination. The *Arabidopsis* genome sequence reveals that plants contain a large number of homologues of SCF components. Whereas only a single SKP1 protein has been identified so far in humans, there are 18 SKP1 orthologues present in the *Arabidopsis* genome. The F-box proteins function in substrate recognition and are the most diverse and specific components of the SCF complex. The *Arabidopsis* genome contains more than 300 F-box–containing proteins (del Pozo and Estelle, 2000; Xiao and Jang, 2000; Andrade *et al.*, 2001; our unpublished data). Cullins also belong to gene families, and in *Arabidopsis*, there are 11 members. This large number of different proteins underscores the potential importance of SCF complexes and regulated protein degradation in various cellular processes in plants.

Yeast complementation tests demonstrated that two distantly related members of the *Arabidopsis* cullin family, AtCUL1 and AtCUL4, have distinct functions in yeast. The AtCUL1 group consists of four additional proteins in *Arabidopsis*. Two of them (At1g59800 and At1g59790) seem unlikely to exhibit the full function of cullins, because they lack a conserved C-terminal region demonstrated to be required for interaction with RBX1/ROC1 (Furukawa *et al.*, 2000; Wu *et al.*, 2000). The other two (At1g43140, At1g02980), however, show high similarities to AtCUL1 and thus might act to some extent in the same or overlapping regulatory pathway.

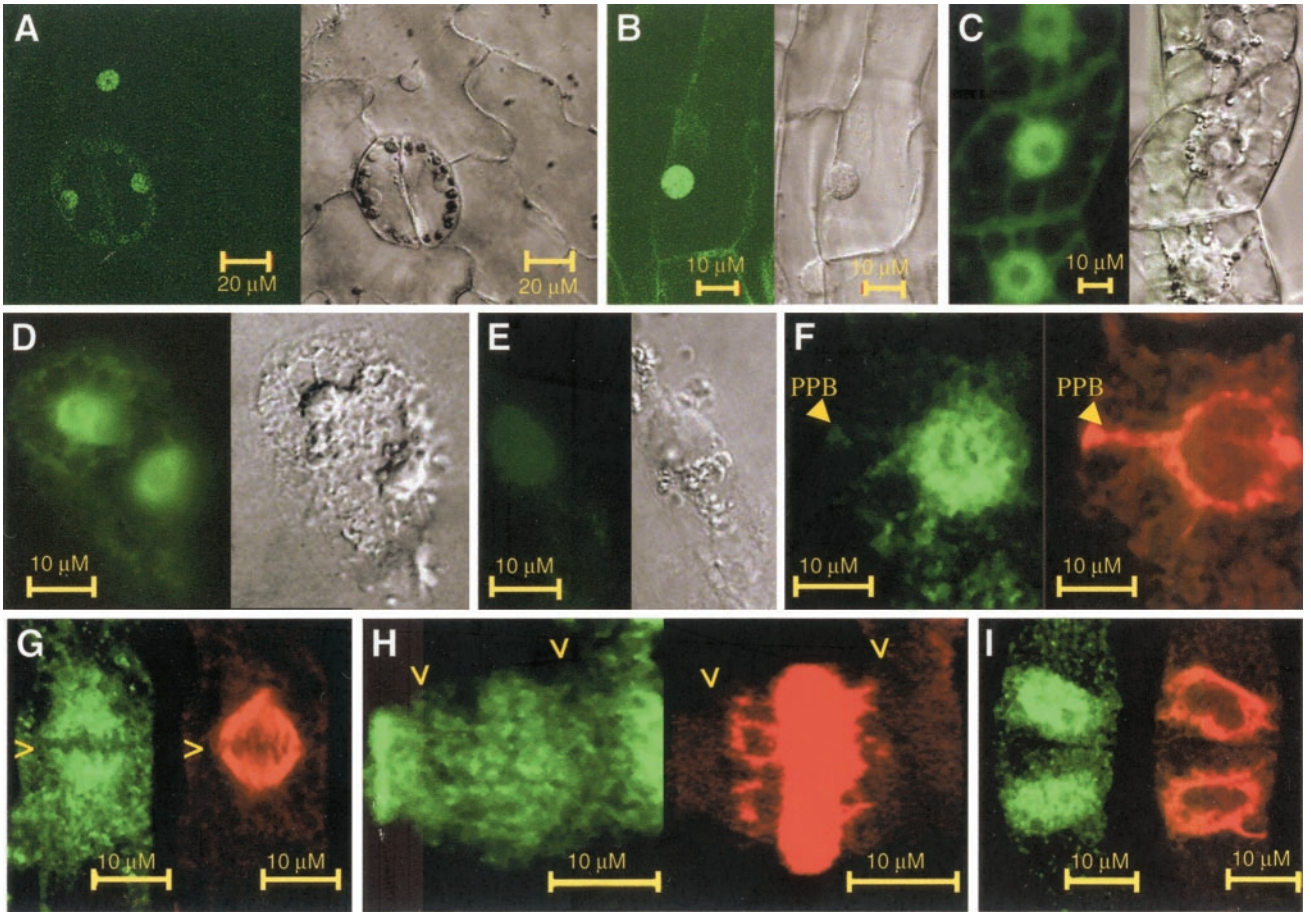

Figure 5. Subcellular localization of AtCUL1. (A) Leaf epidermal pavement and stomata cells of a transgenic tobacco plant expression GFP-AtCUL1. (B) Root cortex cells of a transgenic tobacco plant expression GFP-AtCUL1. (C) Transgenic TBY2 cells expressing GFP-AtCUL1. (D) *Arabidopsis* suspension culture cells showing immunofluorescence after incubation with @AtCUL1 as primary antibody and the Alexa 488-conjugated goat anti-rabbit IgG (Molecular Probes) as secondary antibody. (E) Transgenic TBY2 cells expressing 10×His-tagged AtCUL1 under the control of CaMV 35S promoter were used for immunolocalization with rabbit @AtCUL1 preimmune serum. (F to I) Transgenic TBY2 cells expressing 10×His-tagged AtCUL1 used for coimmunolocalization studies using as primary antibodies the rabbit @AtCUL1 and the mouse @α-tubulin, together with their corresponding secondary antibodies (the Alexa 488–conjugated goat anti-rabbit IgG and the Alexa 568–conjugated goat antimouse IgG, respectively). The fluorescence of Alexa 488 (green, representing AtCUL1) and Alexa 568 (red, representing α-tubulin) was visualized in preprophase (F), metaphase (G), telophase (H), and early interphase (I) cells. > indicates the chromosome position. PPB, preprophase band.

The fact that *AtCUL1* mutants display an arrest in embryogenesis indicates that AtCUL1 is not redundant with any of the other *Arabidopsis* cullin homologues, at least during embryogenesis. There are at least two possible explanations for this. First, different cullins could be required nonredundantly in plant development. In support of this, the close homologues of *C. elegans* cullins CeCUL1 and CeCUL2 are not functionally redundant (Feng *et al.*, 1999). Second, in contrast to the broad expression of the *AtCUL1* in plants, some cullins might be stage- or tissue-specific variants. Consistent with this, no ESTs corresponding to At.g43140 or At.g02980 have been identified thus far. It will be important to examine mutants in different *Arabidopsis* cullin genes to determine the scope of cullin-dependent regulation in plant development.

Subcellular Localization of AtCUL1

Cellular localization studies lend further support to the broad distribution and potential function of AtCUL1. In transgenic tobacco plants expressing GFP-tagged AtCUL1, GFP fluorescence detected in living cells was distributed mainly in the nucleus but also weakly in the cytoplasm (see Figure 5). Similar results were obtained by immunofluorescence (Farràs *et al.*, 2001; this work). In mammalian cells, the CUL1 protein is found in the cytoplasm, in the nucleus, and associated with centrosomes (Freed *et al.*, 1999). Biochemical purification of the centrosome demonstrated that the CUL1 is present in NEDD8-conjugated form. Western blot analysis revealed the presence of the RUB1-conjugated AtCUL1 in *Arabidopsis* plants (del Pozo and Estelle, 1999b; this work). However, plant cells are known to lack distinct centrosomes.

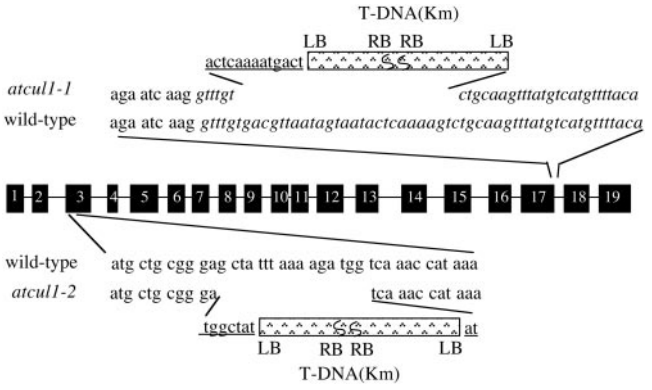

Figure 6. Schematic representation of the T-DNA insertions in the *AtCUL1* gene. The comparison between the genomic and cDNA sequences of the *AtCUL1* revealed that the coding region of the gene consists of 19 exons (black boxes) separated by 18 introns. The junctions between *AtCUL1* and T-DNA are detailed by the representation of the *AtCUL1* exon sequence in triple-nucleotide codon format, intron in italics, and those of unknown origin (corresponding neither to *AtCUL1* nor to T-DNA) in underlined letters. LB and RB indicate the orientation of the left and right borders of the T-DNA, respectively.

Instead, the microtubule nucleation sites are located on the periphery of the nucleus (reviewed in Canaday *et al.*, 2000). At present, it is not clear whether AtCUL1 is colocalized with the nucleation sites. In suspension-cultured cells, AtCUL1 was present mainly in the unconjugated form. In these cells, both GFP labeling and immunofluorescence staining revealed a pattern of AtCUL1 localization similar to that in plant tissues. During mitosis, the patterns of AtCUL1 localization were also quite similar to those demonstrated in *Arabidopsis* plants (Farràs *et al.*, 2001). Therefore, our results do not provide evidence that RUB1-conjugation influences the pattern of AtCUL1 localization. Furthermore, AtCUL1 colocalized with the mitotic spindle. Such colocalization is not reported in other organisms. ASK1 has also been demonstrated to colocalize with the mitotic spindle (Farràs *et al.*, 2001). Together, these results suggest that the AtCUL1-based SCF complexes regulate mitotic processes.

AtCUL1 Is Essential for Embryogenesis

Arabidopsis embryogenesis is initiated upon the delivery of two sperm nuclei to the ovules, one fusing with the nucleus of the egg cell and the other with the central cell nuclei (Berger, 1999; Harada, 1999). The fertilized egg cell is the true zygote, whereas the fertilized central cell divides and forms the endosperm. Development of the embryo is initiated by an asymmetric division of the zygote, producing cells with different fates. The apical cell goes on to produce the embryo proper, and the basal cell generates the hypophysis and the suspensor, a transient organ that plays structural and physiological roles in embryo development. Although large collections of mutants that affect *Arabidopsis* embryogenesis are available, the *atcul1* mutants are the first to be characterized at the molecular level that block the earliest divisions in the developing embryo. Unlike *medea* mutants, which display zygote arrest without affecting endosperm development (Grossniklaus *et al.*, 1998), the *atcul1* mutants are defective in both zygote and endosperm development.

In *C. elegans*, maternal contributions of CUL1 from heterozygous parents suffice for complete development of *cul1*$^{-/-}$ mutants into sterile adults that exhibit the remarkable propensity to undergo extra rounds of cell division in all tissues (Kipreos *et al.*, 1996). Mouse *cul1* mutants show embryo arrest before the onset of gastrulation (Dealy *et al.*, 1999; Wang *et al.*, 1999). *Arabidopsis atcul1* mutants exhibited a much earlier and stricter arrest of embryogenesis, before the first cell divisions after fertilization. This difference may reflect different contributions of CUL1 from heterozygous maternal tissues (mRNA or protein) to zygote development and/or different requirements for protein degradation during embryogenesis in each organism. Consistent with its

Table 1. F1 mutant progeny

	Progeny				Hypothesis			
					χ^2			
Genotype	No. of plants Km-resistant (R)	No. of plants Km-sensitive (S)	R/S	df	R/S = 1	R/S = 2	R/S = 3	1< R/S[a] <2
atcul1-1+/− selfing	14183	8082	1.75	64	1304.95	**166.48**	1572.8	**36.23**[b]
atcul1-2+/− selfing	3187	2100	1.52	18	228.67	**102.86**	617.91	**13.47**[b]
atcul1-1+/− × *atcul1-2*+/−	118	69	1.71	2	12.83	**1.07**	14.12	**0.02**[b]
atcul1-2+/− × *atcul1-1*+/−	71	56	1.27	2	**1.77**	6.61	24.69	**0.29**[b]
atcul1-1+/− × wild-type	279	356	0.78	4	**9.69**	148.03	327.26	
wild-type × *atcul1-1*+/−	235	348	0.67	4	**21.51**	183.21	372.85	
atcul1-2+/− × wild-type	67	144	0.47	2	**28.09**	115.73	210.46	
wild-type × *atcul1-2*+/−	135	149	0.91	2	**0.69**	46.77	114.25	
atcul1-1+/− (pAtCUL1)	3216	1102	2.92	12	1034.95	118.37	**1.68**[b]	
atcul1-2+/− (pAtCUL1)	745	244	3.05	4	252.78	33.39	**0.06**[b]	

[a] Homozygote lethal (R/S = 2) and with correction of reduced inheritance determined by back-crosses with wild-type.
[b] $p > 0.5$.

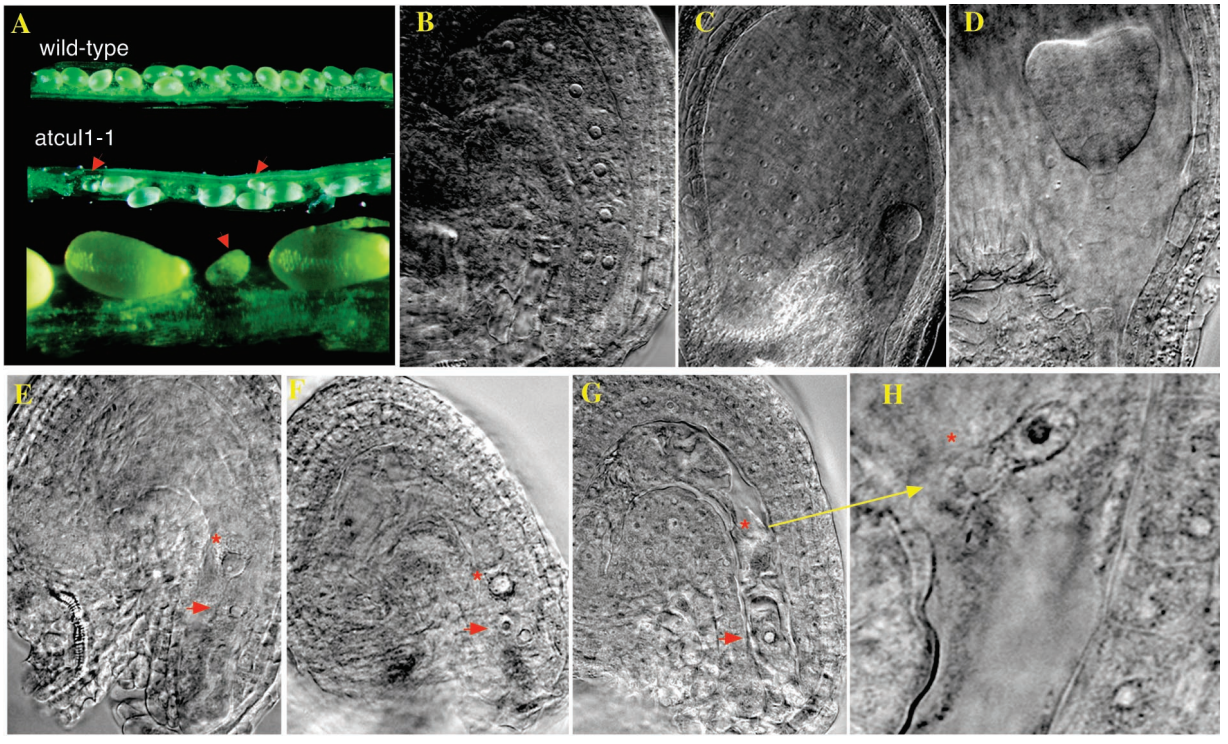

Figure 7. Phenotypes of *atcul1* mutants. (A) Open siliques from self-pollinated wild-type and *atcul1–1* mutant plants. Arrows indicate the developmental arrested siblings. (B–H) Differential interference contrast images of cleared ovules from self-pollinated *atcul1–1* mutant plants. (B–D) Embryogenesis of developing siblings (containing wild-type or heterozygous zygotes and endosperm) at preglobular (B), globular (C), and heart (D) stages. (E–H) Ovules of arrested siblings from siliques of globular to early heart age. The arrested zygote (arrow) and endosperm (asterisk) cells contain 1 or 2 nuclei each.

essential function, the *AtCUL1* gene is highly expressed, at both transcript and protein level, in the embryos.

Mutations in genes encoding F-box proteins as well as ASK1 have been identified previously in *Arabidopsis*. These mutants differ from *atcul1* in that they are viable as homozygotes. The homozygous *ask1–1* mutant is male sterile, indicating a more strict requirement for ASK1 in male gametogenesis (Yang *et al.*, 1999). Genetic analysis demonstrated that the heterozygous *atcul1* mutant plants produced functional *atcul1*$^{-}$ pollen and ovules, and microscopic examination revealed that both male and female gametogenesis are morphologically normal (data not shown). This makes *AtCUL1* functionally distinct from genes specifically required for gametophyte biogenesis (Yang and Sundaresan, 2000). Nevertheless, a reduced inheritance in both male and female *atcul1*$^{-}$ gametophytes was observed, suggesting that the gene does have a function in the gametophyte. The severe phenotype of the *atcul1* mutants compared with the previously characterized mutants in the other SCF components strongly suggests that AtCUL1 forms multiple SCF complexes with different ASKs and F-box proteins, which are ultimately required for plant cell division and embryogenesis. At present, only the SCF^{TIR1} complex containing the AtCUL1, ASK1, and TIR1 (Gray *et al.*, 1999) and SCF^{COI1} containing the AtCUL1, ASK1, and COI1 (D. Xie, personal communication) have been demonstrated. Other SCF complexes remain to be biochemically characterized.

SCF Pathway, Cell Division, and Embryogenesis

The best characterized plant SCF complex is SCF^{TIR1}, which is involved in auxin signaling (del Pozo and Estelle, 1999a; Gray *et al.*, 1999; Dharmasiri and Estelle, 2002). It is well known that auxin plays a crucial role in cell division and embryogenesis (Harada, 1999; Chen *et al.*, 2001). Targets of SCF^{TIR1} include at least some of the large family of transcriptional regulators, the Aux/IAA proteins, involved in auxin response (Gray *et al.*, 2001). The important function of SCF^{TIR1} in plant development is further evidenced by the finding that the COP9 signalosome, involved in photomorphogenesis, regulates the RUB1 conjugation of AtCUL1 and consequently the SCF^{TIR1}-mediated auxin response (Schwechheimer *et al.*, 2001). However, because of the absence of embryo arrest phenotype in the loss-of-function mutant *tir1*, the SCF^{TIR1} pathway alone does not suffice to explain the phenotype of the *atcul1* mutants. It is likely that several SCF complexes are defective in *atcul1*$^{-/-}$ cells and that the accumulation of multiple misregulated target proteins is responsible for the embryo arrest phenotype of the *atcul1* mutants.

In view of the emerging roles of SCF pathways in many cellular processes, numerous substrates are likely to accumulate in cul1$^{-/-}$ cells, one or more of which may account for the embryogenesis arrest. The SCF pathway plays an essential role in the cell cycle control. In yeast, SCF com-

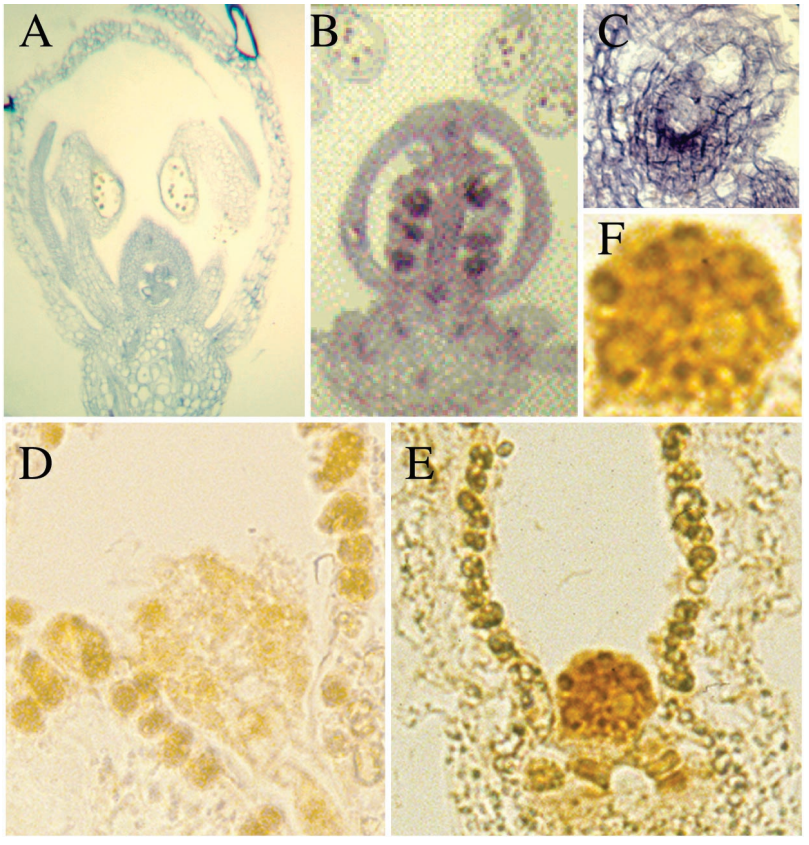

Figure 8. Expression of *AtCUL1* in embryos. (A) Negative control of in situ hybridization with *AtCUL1* sense probe in a flower section. (B) A flower section probed with the *AtCUL1* antisense probe showing strong staining in ovules of a silique. (C) Close-up of an ovule from (B) showing staining of the embryo. (D) Negative control of immunostaining with the preimmune serum of an ovule containing globular embryo. (E) Immunostaining of an ovule containing globular embryo with @AtCUL1 antibodies, showing strong staining of the embryo. (F) Close-up of the stained embryo from (F) showing strong staining in the nuclei.

plexes function in both G_1/S and G_2/M transitions. *cdc53* mutants are defective for the G_1-to-S phase transition, because of accumulation of the CKI SIC1, whose degradation depends on the SCF^{CDC4} complex (Schwob *et al.*, 1994). In cells in which *SIC1* has been deleted, *cdc53* mutants undergo a block at G_2-to-M transition, because of accumulation of the CDK-inhibitory kinase Wee1 (a negative regulator of G_2/M transition), whose degradation depends on the SCF^{Met30} complex (Kaiser *et al.*, 1998; Michael and Newport, 1998). Known substrates of SCF complexes in mammals include cell cycle regulators, such as the CKI ($p27^{KIP1}$), G_1-type cyclins (cycD and cycE), and the transcription factor E2F, as well as the signaling protein IκBα (reviewed in Krek, 1998; Deshaies, 1999; Maniatis, 1999; Tyers and Jorgensen, 2000). Although the G_1-type cyclin E accumulates in the arrested mice $cul1^{-/-}$ embryos, it seems unlikely that this accumulation should be the cause of arrest, because cells can tolerate high levels of cyclin E expression (Wang *et al.*, 1999). Thus, the reason for the developmental arrest of $cul1^{-/-}$ embryos in mice remains unknown.

Although homologues of cyclin E have not been identified in plants, orthologues of Wee1, CKI, cyclin D, and E2F are found in plants (Shen, 2001a). The plant D-type cyclins contain the conserved PEST motif, suggesting that they are degraded through the SCF complex, similar to mammalian cyclin D. Recent transgenic studies reveal that ectopic expression of cyclin D and CKI dramatically affects plant development (Riou-Khamlichi *et al.*, 1999; Cockcroft *et al.*, 2000; Wang *et al.*, 2000; De Veylder *et al.*, 2001). It is reasonable to speculate that accumulation of such cell cycle regulators in $atcul1^{-/-}$ cells might profoundly affect cell division. In addition, colocalization of AtCUL1 with the mitotic spindle suggests that SCF complexes might be involved in the control of chromosome segregation. Consistent with a role of the ubiquitin pathway in cell cycle control, several other mutants in genes encoding a proteasome subunit and ubiquitin-specific proteases are also embryo-lethal or affect cell divisions in *Arabidopsis* (Doelling *et al.*, 2001; Smalle *et al.*, 2002; Tzafrir *et al.*, 2002).

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